Some aqueous media for preserving algae for class material.

W. A. SETCHELL AND W. J. V. OSTERHOUT.

There are ordinarily two difficulties in the way of introducing a careful study of the various marine and fresh water algæ into a course in cryptogamic botany. The first of these is the obtaining of the material, and the second is preserving the material which may be obtained in such fashion that it can be placed before the student in a condition to be readily examined and studied with nearly as satisfactory results as those afforded by the fresh material of the same forms.

The first difficulty can be overcome more or less readily. Fresh water species are more or less abundant in our ponds, brooks and rivers, and the increasing facility of access to the sea brings the marine forms within the reach of many. Especially do the facilities offered by the marine laboratories, such as those at Cold Spring Harbor, N. Y., at Woods Hole, Mass., and at Pacific Grove, Calif., afford an opportunity for the teacher of botany not only to become acquainted with the algal forms and their use in the class room, but also to obtain and preserve a good supply of desirable species in the very best condition possible. Under the auspices of the Marine Biological Laboratory at Woods Hole, a Department of Laboratory Supply has been in successful operation for several years, and from it all necessary botanical material may be very satisfactorily and economically obtained.

The old method of preserving in strong alcohol shrivelled the specimens to such an extent that the use of strong swelling reagents (alkalies or acids) was necessary to show anything like the proper degree of detail of structure, and while these methods were good for the ordinary tougher species, and when applied by students of some experience, yet they were very unsatisfactory when applied to the more delicate forms or when used by the more inexperienced manipulators.

The use of the weaker alcohol, 50–70 per cent. according to the particular specimen to be preserved, was better, yet proved decidedly unsatisfactory for the more delicate forms.

The ordinary English method of fixing in a saturated solu-

tion of picric acid and preserving in strong alcohol is a very good one, especially for specimens to be imbedded in paraffin or for special work in connection with particular problems. Better still is fixing in some special solution such as a saturated solution of picric acid, 0.5–1 per cent. chromic acid, Perenyi's fluid, Hermann's mixture, etc., and transferring through the ordinary grades of alcohol, or by dialysis, up to 70 per cent. strength and preserving in that.

Such material is in excellent condition for imbedding in paraffin or celloidin, but for the ordinary class work, for manipulation by the student himself, the specimens must generally be transferred again to water.

But the preparation by these methods of material for a large class is often a considerable task. The more delicate forms too are seldom in a thoroughly satisfactory condition.

It has been found to faciliate the class-work on all the cryptogams very much to use freezing methods in the preparation of sections for the class, and either to have the sections cut by an assistant or by different members of the class at different times. A description of a convenient freezing device and methods of imbedding in aqueous media will be published by one of us in the next number of this journal.

Freezing methods and the preservation of natural form and size of the different parts with as little change as possible have rendered it very desirable that aqueous media be employed if possible for preserving fluids.

A number of fluids have been subject to experiment by the writers for about three years, particularly upon the abundant materials of all groups of algæ obtained at the Marine Biological Laboratory at Woods Hole, Mass. It is thought by the writers that these notes of their experience, while containing nothing especially new, may serve as useful hints to those who have before them the problem of providing and preserving cryptogams for laboratory purposes.

Chrome alum.

This substance was used by Guignard[1] for fixing various Laminariaceæ for the purpose of investigating the structure and development of the mucilage ducts. Later it has been tested at the Biological Station at Helgoland by Lotsy[2] upon

[1] Ann. Sci. Nat., Bot. VII. **15:** 1–46. 1892.
[2] Bot. Centralblatt **60:** 15–16. 1894.

the red algæ particularly as to the preservation of the cell-structure.

The writers have used one per cent. chrome alum in either distilled water or sea water carefully filtered through sand, according to the different habitat, for about four years. The algæ, carefully selected and washed free from dirt and debris, have been placed in it at once and preserved in it until needed for examination. The cell structure is well preserved in all cases. Very little washing is needed afterwards to allow staining by any of the ordinary staining reagents. Gelatinous intercellular substances, whether soft or more cartilaginous, are rendered firm but not especially opaque by treatment with it. Cyanophyceæ, Chlorophyceæ, and Rhodophyceæ do very well indeed. Phaeophyceæ, almost without exception, are rendered brittle in a short time, but while this renders them troublesome to manage, yet specimens prepared in this way and soaked out in water are excellent for study by crushing methods. It is the intercellular substance that is rendered brittle and such forms as species of Leathesia, Mesogloia, Laminaria, etc., when crushed, spread out and show the cell structure and cell arrangement in a very satisfactory fashion. The color is not retained perfectly, but is ordinarily retained more than by any other of the media we have tried.

The Chlorophyceæ lose all of their green, or nearly all. The Cyanophyceæ and Rhodophyceæ often retain considerable (especially if kept away from the light), generally at least enough to assist materially in the examination of the chromatophores, while the Phaeophyceæ lose very little of their intensity. Specimens preserved in chrome alum must be kept in glass-stoppered jars, carefully closed, as the solution is liable to become invaded by various molds. A little finely divided camphor-gum at the top will prevent this, as will also a small quantity of formalin. Chrome alum solution has a certain corrosive action upon metals, so that metal tops to the preserving jars should be avoided, and specimens to be sectioned free-hand or with the freezing microtome methods, should have at least the greater part of the alum removed by washing.

One per cent. chrome alum is also an excellent preserving fluid for use with fungi of the various groups, for the mosses, for ferns and for flowering plants, better in all cases than the strong alcohol commonly used, but probably not superior to

the various percentages of formalin, except in the case of gelatinous forms. Spirogyra cells keep well in 1 per cent. chrome alum, the chromatophores, pyrenoids, nuclei and protoplasmic sac and threads showing very well indeed. Specimens kept in a cork-stoppered bottle in chrome alum showed a very distinct dark steel-blue stain affecting the nucleolus most, the nucleus and the chromatophores, and this remained after washing in water, dehydrating, and mounting in Canada balsam.

With chrome alum, as well as all other preserving media, a fairly large proportion of fluid should be used.

Formalin.

Formalin, formalose, or 40 per cent. formaldehyde, according to the trade name, has in the last two years become very popular with both zoologists and botanists. It is not necessary for us to go into the literature, but we have found that the 1–2 per cent. solution of the formalin (1–2^{cc} formalin in 99–98^{cc} distilled water or sea water) makes a solution sufficiently powerful to kill, fix, and preserve any ordinary vegetable tissue. While the color fades more rapidly than with chrome alum, the cell contents are preserved equally well. For Phæophyceæ, a 2 per cent. formalin solution is the very best fluid which we have tried. Cyanophyceæ preserve their structure but not the gelatinous matrix so well, since this is liable to shrink under the influence of formalin. Delicate Rhodophyceæ, such as Griffithsia, Callithamnion, Dasya, etc., keep their full form better than in any other fluid. Chlorophyceæ do equally well. Formalin solutions containing organic materials become acid after a short time and this may tend to alter the cell-contents or the intercellular substance slightly, but in preparations kept for nearly two years this is not sufficiently marked to be especially noticeable. Formalin in the same percentages works excellently for fungi and the higher plants. Toadstools are preserved in their natural shapes and in more or less of their natural colors according to the species.

Camphor water.

Camphor-gum is sparingly soluble in water, but the solution is very prejudicial to the life of microorganisms. Camphorated water is very useful when considerable collections have been made and cannot be examined for several hours.

In such cases small pieces of camphor-gum strewn in the water help to keep the algæ from putrefying until they can be studied or properly sorted and preserved. Formalin is useful also for this purpose, but the acidity produced changes the color quicker than is the case in camphorated water. For preserving Cyanophyceæ, camphor water keeps the cell structure well if present in large volume, proportional to the amount of material, but the coloring matter is soon dissolved. Chlorophyceæ, Phaeophyceæ, and Rhodophyceæ, if well sorted and cleaned, are well preserved in abundance of the fluid, even the finer details of cell structure being preserved perfectly. But perhaps the most important use of camphor water is to preserve specimens already fixed by other fluids. Specimens of the larger Rhodophyceæ, killed and fixed in concentrated aqueous solution of picric acid are preserved to especial advantage in camphor water; as one of us has experienced in special work upon *Rhabdonia tenera* Ag.

Summary of results.

Cyanophyceæ are best prepared with a solution containing 1 per cent. chrome alum and 1 per cent. formalin. This solution renders the gelatinous sheath and matrices firm, keeps the cell contents in a very natural condition, and retains in most cases the colors in their ordinary tints. 1–2 per cent. formalin solution preserves the cell contents very well indeed, but does not keep the color well, or the softer gelatinous sheaths and matrices. Camphor water is not very favorable for many blue-greens. Many species must needs be preserved in mass, and are associated with many bacteria and the camphor solution is hardly strong enough to wrestle successfully with the latter.

Chlorophyceæ are very satisfactorily preserved in any of these media. Chrome alum is to be preferred in most cases, but some species are rendered very brittle as, *e. g.*, membranaceous forms like Ulva Lactena. Such forms are of course better if placed in simple formalin solution.

Phaeophyceæ do well when placed immediately in 1 per cent. formalin in sea water. The larger forms are better fixed in 1 per cent. chrome alum for a few hours (3–6) and then preserved in 2 per cent. formalin solution or camphor water. But specimens for crushing may be allowed to remain indefinitely in the chrome alum solution.

Rhodophyceæ. The coarser forms may be put into any one of the three solutions and be in very excellent condition; chrome alum preserves more color than formalin or camphor water. For the finer study, specimens are best left in a concentrated solution of picric acid in sea water for twenty-four hours, then washed, preferably in sea water, for about twenty-four hours more, and preserved in camphorated sea water. Such genera as Nemalion, Champia, Rhabdonia, Cystoclonium, etc., respond best to this treatment. Delicate species need very careful consideration. Griffithsia Bornetiana is a most delicate species and, preserved in almost any way, collects itself together into a shapeless mass; the cells lose their shape, and it becomes a very uninviting object for study. But placed in 2 per cent. formalin in sea water with plenty of fluid so as not to be crushed, the cells keep their shape and the whole plant presents a life-like appearance as far as form goes. The color of course departs. The same thing is true of various species of Callithamnion, such as C. Baileyi, C. Borreri, C. seirospermum, etc. Dasya elegans has a way of dropping its hairs on being preserved, and the more delicate species of Polysiphonia break up into short pieces, but either formalin or chrome alum will prevent this if the specimens are fairly fresh when put into the preserving solution.

Berkeley, Cal., and *Providence, R. I.*